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Dactylis glomerata growing along a light gradient in the central Appalachian region of the eastern USA: III. Nonstructural carbohydrates and nutritive value

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Abstract

Microsite conditions influence plant development and resource allocation of Dactylis glomerata L. (orchardgrass), a traditional pasture species with potential as an understory crop in woodlots. A field experiment was conducted to determine how open (O), shaded woodland (W) and open-to-shaded woodland transition zone (E_O, E_W) microsites influenced the quantity and distribution of nonstructural carbohydrate (TNC) and crude protein (CP) among plant parts of defoliated orchardgrass. Plants established in spring (SP) or late summer (LS) were clipped each time mean sward height reached 20 cm. Microsite conditions influenced nutritive value of herbage. Nutritive value was acceptable when not more than 45–50% light attenuation (as a function of shading by nearby trees) occurred relative to open pasture. Twice as much TNC accumulated in stembases of LS compared to SP plants. Concentrations of TNC were least in plants growing at W, regardless of planting time. Stembase TNC depletion occurred in SP plants, regardless of microsite, and LS plants growing at W. CP concentrations were greater in herbage from W compared to O sites, suggesting the N needs of the plant were met with minimal fertilizer N inputs. The ratio of C:N and thus herbage energy expressed as total digestible nutrients (TDN), relative to CP declined as shade increased. Increased protein content is offset by lower fiber, lower nonstructural carbohydrate and the possibility of reduced preference by grazers. Nutritive value is improved by modest amounts of shade relative to plants growing in full sunlight, and allowing cool temperate origin grasses to vernalize is beneficial in terms of productivity, nutritive value, and persistence.

Introduction

Complex topography and associated plant communities in hilly terrain create a variety of microsites that influence spatial and temporal boundaries of forage production. Land-use effi-

ciency benefits when the mosaic of pasture and woodland, typical of hill-land regions, is used for silvopasture. Devkota and Kemp (1999) reviewed economic and ecological features of silvopastoral systems in temperate regions and found that successful and productive systems depended on shade tolerant forages. Shading often depressed herbage mass, but nutritive value was considered greater in shade-grown than full-sun herbage (Deinum et al.

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1968; Kephart and Buxton 1993; Lin et al. 2001; Burner and Belesky 2004). Variation in herbage mass and nutritive value among the shaded and open components of silvopastoral systems complicates grazing management strategy.

The combined effects of shade and defoliation alter productivity, seasonal distribution and photosynthate partitioning in *Dactylis glomerata* L., hereinafter referred to as orchardgrass (Belesky 2005a). Changing physiological efficiencies (Belesky 2005b) caused by the simultaneous stresses of shade and defoliation affect accumulation of nonstructural carbohydrates and N, and as a result, nutritive value.

Shade-grown, cool-temperate origin grasses allocate N to leaves as an adaptive mechanism to help optimize light acquisition. Unassimilated N, occurring as nitrate along with depressed levels of TNC in shade-grown herbage (Deinum et al. 1968; Chiavarella et al. 2000) could compromise nutritive value. Available herbage TNC concentration was positively associated with improved dietary protein utilization in the rumen, and increased selection and intake of TNC-rich forage by grazers (Chiavarella et al. 2000; Mayland et al. 2000). Imbalances in relative amounts of herbage energy (low TNC in this case) and N (high CP) could lead to off-flavors influencing consumer acceptance of pasture-raised beef (Lane and Fraser 1999), or environmental concerns related to water quality associated with excess N excretion.

Environmental, seasonal, diurnal and ontogenetic changes influence patterns of TNC accumulation. In general, polymers of fructose, or fructans, accumulate in stembases of cool-temperate origin grasses when photosynthate production exceeds carbohydrate translocation and utilization rates. Fructan can account for as much as 40% of plant dry mass and influence the pattern of relative carbon allocation among plant structures (Cairns et al. 1999). Stored TNC is involved in environmental and competitive stress tolerance (Cheplick and Chui 2001), and also provides energy for regrowth after defoliation; however, defoliation frequency (brief interval between events) or intensity (minimal plant residual height after an event) can impair TNC replenishment.

Canopy defoliation based on leaf development integrates morphological and phenological attributes of forage plants with management and environment, and generally optimizes leaf pro-

duction. The outcome is sustained herbage productivity, sward persistence, and somewhat more predictable nutritive value and grazing livestock performance (Hodgson 1990). Defoliation based on mean sward height (e.g. leaf elongation) might not apply for shaded sites in silvopastoral systems since leaf elongation is a response to low irradiance and not simply an inherent morphological characteristic of the plant (Belesky 2005a). The objective of this experiment is to determine the quantity and distribution of TNC and CP as a function of light attenuation for defoliated orchardgrass and consider these in terms of dry matter allocation among plant structures (Belesky 2005a) and mechanisms of dry matter production (Belesky 2005b). Our goal is to use this information to develop canopy management strategies to produce orchardgrass leaf dry matter (DM) with the nutritive value required by growing livestock in silvopastoral systems in humid regions.

Materials and methods

Growing conditions

Procedural details are presented in Belesky (2005a). Briefly, orchardgrass, cultivar Benchmark, (early flowering) was sown (100 seed pot^{-1}) in 2.5 l pots containing a mixture of four parts soil (Lily, fine-loamy, siliceous, semi-active, mesic, Typic Hapludult) and three parts sand. Container-grown plants (with the container bottoms removed) were used to minimize site and soil related effects on germination, growth and nutrient availability. Dolomitic limestone (2 mg ha^{-1}) and hydrated lime (1 mg ha^{-1}) were applied to raise soil pH to 6.3 and added along with 35 kg ha^{-1} N, P and K as commercial fertilizer (Peters Professional® All Purpose 20-20-20 from W. R. Grace & Co., Fogelsville, Pennsylvania, USA) applied to all pots when placed at the respective sites. Plants were fertilized with an annual split application totaling 100, 60 and 120 kg ha^{-1} N P and K with one-third of the total applied in May, early July and mid August in each year. Plants were watered (~500 ml per pot) when precipitation was minimal during the growing season.

Plants were grown for 6 weeks in a controlled environment, with a 12 h photoperiod, 24/18 °C light/dark temperature and 55% relative humidity.

Pots were placed outside the glasshouse in a non-shaded area for 2 wk prior to placement at microsites in early May (spring planting, SP) or mid August (late summer planting, LS) of 2001. Microsites (81°7′ E; 37°45′ N; 765 m elev.) included an open (O) unobstructed pasture, a wooded (W) site dominated by *Quercus* spp. with 89.8% light attenuation relative to O, and two south-facing, edge (E) zones E_O and E_W with a growth interval mean of 30 and 56.4% light attenuation from similar tree species in W, respectively. The W and O sites were about 60 m apart and the transition sites midway between.

Sample collection and analysis

Baseline data were collected from nine pots for SP and LS immediately prior to planting. All plants were clipped to a 5 cm residual height. Three replicates were collected each time mean plant height reached 20 cm, with leaf (above 5 cm), stembase (soil surface to 5 cm) and root lyophilized and mass determined. The SP plants were harvested in the year in which they were planted and LS plants in the growing season after planting.

Lyophilized samples were ground in a Cyclone mill (Udy Corporation, Fort Collins, Colorado) to pass a 0.5 mm screen. Nitrogen and carbon were determined by total combustion (Carlo Erba EA 1108 CHNSO analyzer. Fisons Instruments, Beverly, MA), and nitrates by ion-selective electrode (Consalter et al. 1992). Nonstructural carbohydrates were determined by an automated hydrolysis method (Denison et al. 1990). Fructan polymerization analysis was conducted according to methodology reported in Chatterton and Harrison (1997). We estimated average degree of polymerization values by hydrolyzing an aliquot of the aqueous extract with 1 N HCl for 20 min at 70 ° C and measuring glucose and fructose by ion exchange chromatography. The hydrolysate included sucrose and monosaccharides in extracts, as well as hydrolyzed fructan. Thus, average DP values underestimate the true mean degree of polymerization (DP) of fructans but illustrate relative differences that may occur among treatments.

Dried tissue samples were analyzed for *in vitro* organic matter disappearance (IVOMD) by a procedure developed by Moore (1970). The IVOMD procedure used rumen fluid obtained from rumen-cannulated steers (*Bos taurus*) offered orchardgrass – alfalfa (*Medicago sativa* L.) hay. Computations for nutritive value included, crude protein as CP g 100 g^{-1} = (total N g 100 g^{-1} × 6.25) and metabolizable energy of feed (ME) as ME (MJ kg^{-1} DM) = 0.0157 (IVOMD) (AFRC 1993). Total digestible nutrients (TDN) were calculated from ME data (NRC 1996).

Statistical analysis

Data were analyzed using SAS MIXED procedure with light attenuation (sites O, E_O, E_W or W), and harvest as fixed effects and replication and interactions with replication as random in the model. Analyses were performed separately on each planting time. Significant treatment mean differences indicated by *($p < 0.05$), **($p < 0.001$), ***($p < 0.001$) or 'ns' (not significant) throughout the text.

Results and discussion

TNC in leaf and stembase as a function of light attenuation

Light attenuation did not influence TNC concentration in leaves of SP plants (Figure 1a); however, stembase TNC was influenced by light level and was greatest in plants grown at O and least at W sites (Figure 1b). Concentrations in herbage grown at O did not differ from E_O and E_W. Leaf (Figure 1a) and stembase (Figure 1b) TNC concentrations of LS plants were greater at O than W microsites. Low TNC concentrations in leaves and stembases of SP plants characterize an establishing plant allocating energy to structural increase rather than storage. Accumulation of TNC in leaves of LS plants at O and E_O sites relative to E_W and W suggests that photosynthate production and stembase TNC storage were optimal. The SP plants allocated photosynthate to roots and stembases (Belesky 2005a), so the likelihood of TNC accumulating in leaf was minimal. The LS plants had well developed root systems and few but relatively large tillers (Belesky 2005a) that apparently could sustain regrowth and allow TNC to accumulate in the leaf. The differences between SP and LS plants might be a function of plant age.
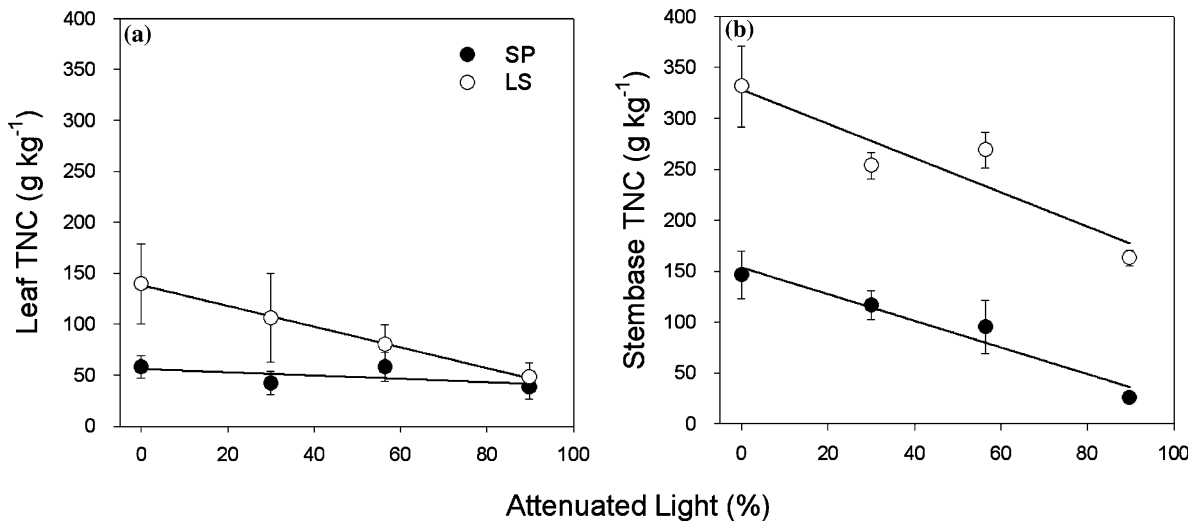
Figure 1. (a) Leaf and (b) Stembase total nonstructural carbohydrate (TNC) as a function of microsite for spring (SP) and late-summer (LS) plantings. Values are mean and standard error of the mean of five harvests for each microsite of spring (SP), three harvests for open (O) and edge-open (E_O), four for edge-woods (E_W) and five for woods (W) for late-summer (LS) planted orchardgrass. Test $\beta_1 = 0$: (a) SP, -0.2240 ns, $r^2 = 0.80$; LS, -1.1459*** $r^2 = 0.99$; (b) SP, -13.0423* $r^2 = 0.95$; LS, -16.8409 ns, $r^2 = 0.86$.

Change in stembase TNC

The change in TNC occurring in stembases across a growing season provides some insight into TNC dynamics in the whole plant in terms of accumulation and depletion as a function of repeated clipping and light attenuation. Stembase TNC depletion occurred across the growing season for SP plants at all microsites and did so the most for plants growing at W (Figure 2). Stembase TNC of LS plants increased at O, was near equilibrium in plants grown in E_O or E_W sites, and declined at W (Figure 2). Storage occurs when the production of photosynthate exceeds requirement for growth and maintenance (Lemaire and Millard 1999). Repeated defoliation of juvenile plants, represented by SP plants in this experiment, did not lead to storage of TNC, whereas LS plants were able to store TNC despite being subjected to the same degree of removal. Clipping orchardgrass growing in shaded sites prior to vernalization might compromise stand establishment and persistence. Accumulated TNC in juvenile plants is used to form new leaves to restore light capture capability rather than consolidate resources in a larger plant. Vernalized plants can form new tillers or seed and might have different allocation strategies in terms of TNC storage than juvenile plants. Numerous experiments show that tiller initiation is related to TNC reserves (Fulkerson and Donaghy 2001)

Leaf and stembase sucrose as a fraction of TNC

Sucrose in the leaf influences photosynthesis and carbohydrate storage (van der Werf and Nagel 1996). Sucrose concentrations in defoliated perennial ryegrass were as much as 70% of the TNC occurring in elongating basal portions of leaves during regrowth intervals (Morvan-Bertrand et al. 1999). The fraction of TNC occurring as sucrose was not influenced by light attenuation in leaf or stembases of SP plants (Figure 3). Sucrose represented about 25% of leaf and 30% of stembase TNC in SP plants. The pattern suggests that sink activity (e.g. allocation of photosynthate to root and stembase) is high with relatively little accumulation of sucrose or polymeric carbohydrate forms such as fructan in the stembase (Pollock et al. 2003). The fraction of TNC occurring as sucrose in LS plants decreased in leaves and stembases as light attenuation increased (Figure 3). Sucrose
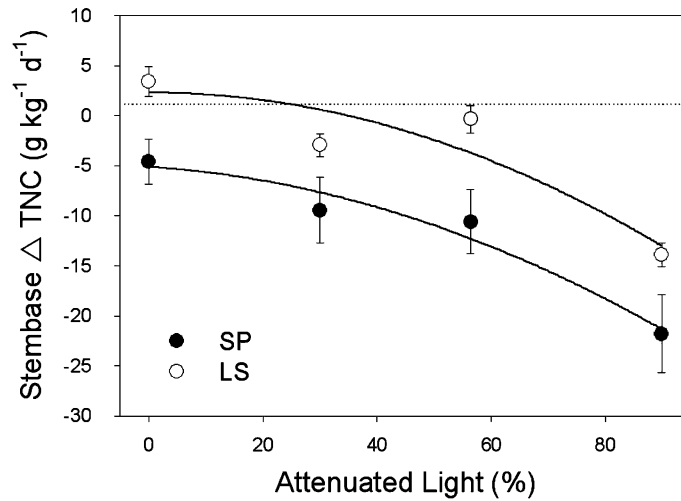
Figure 2. Change in stembase TNC (Δ TNC) as a function of microsite for spring (SP) and late-summer (LS) plantings. Values are mean and standard error of the mean of five harvests for each microsite of spring (SP) and three harvests for open (O) and edge-open (E$_O$), four for edge-woods (E$_W$) and five for woods (W) for late-summer (LS) planted orchardgrass. Test $\beta_1 = 0$: SP, −0.0365 ns $r^2 = 0.96$; LS, 0.0003 ns $r^2 = 0.84$.
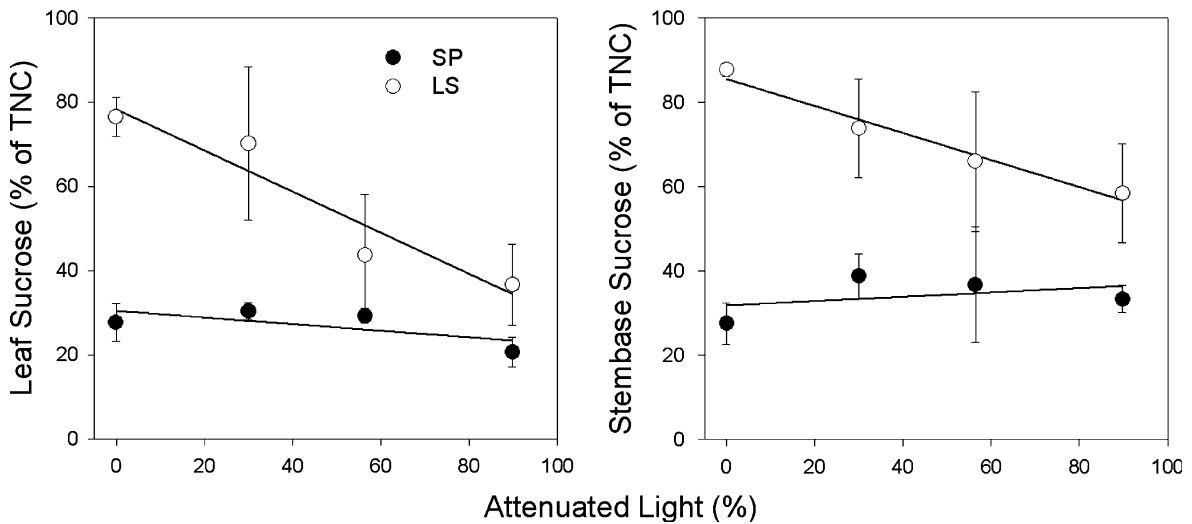

Figure 3. (a) Leaf sucrose as a percent of total nonstructural carbohydrate (TNC); (b) stembase sucrose as a percent of TNC as a function of microsite for spring (SP) and late-summer (LS) plantings. Values are mean and standard error of the mean of five harvests for each microsite of spring (SP) and three harvests for open (O) and edge-open (E$_O$), four for edge-woods (E$_W$) and five for woods (W) for late-summer (LS) planted orchardgrass. β value for mean slope effect Test $\beta_1 = 0$: (a) SP, −0.0769 ns $r^2 = 0.46$; LS, −0.4873* $r^2 = 0.91$; (b) SP, 0.0516 ns $r^2 = 0.16$; LS, −0.3221* $r^2 = 0.97$.

represented 80 (leaf) and 90% (stembase) of TNC for LS plants growing at O and 40 (leaf) and 60% (stembase) of TNC for plants growing at W. The fraction of TNC attributable to suc-rose could be associated with the average DP value of fructans that help sustain regrowth, or contribute to persistence and cold tolerance (Moriyama et al. 2003).

Degree of polymerization as a fraction of water-soluble carbohydrate

The DP values presented include sucrose and monosaccharides, as well as hydrolyzed fructans that represent differences attributable to light conditions and major plant structures. Average DP values were less for SP than LS plants (Figure 4). The difference reflects greater TNC concentrations, and involvement of processes associated with maturity and fructan metabolism. The average DP for SP plants was greater at O compared to W, regardless of plant part. Average DP values for SP plants ranged from 1 (W) to 3 (O) for leaf, 4 (W) to 11 (O) for stembase and 1 (W) to 4 (O) for roots (Figure 4). Average DP value for LS plants ranged from <1 (W) to 6 (O) for leaf, 8 (W) to 15 (O) for stembase and 3 (W) to 6 (O) for root.

Pollock et al. (2003) suggest that photosynthetic carbon fixation operates to balance regeneration of ribulose bisphosphate carboxylase (E.C. No. 4.1.1.39). They proceed to explain sucrose and fructan accumulation as a product of processes that increase photosynthesis or reduce sink demand, such as defoliation and root loss. Repeated defoliation (e.g. grazing) events are likely to place heavy demand on accumulated carbohydrate supply. Partial shade conditions in conjunction with repeated defoliation are likely to retard fructan accumulation and impact sustained regrowth capability.

Leaf nitrogen as crude protein and NO_3–N

Leaf N expressed as CP, was least in plants at O and greatest at W, irrespective of planting time (Figure 5a). Concentrations in SP plants ranged from 20 (O) to 32 g 100 g^{-1} (W), whereas concentrations ranged from 13 (O) to 18 g 100 g^{-1} (W) for the LS planting. Leaf NO_3 concentration of SP plants ranged from 2 mg g^{-1} DM (O) to 7 mg g^{-1} DM (W) as light decreased (data not shown). Nitrate concentrations were negligible in LS plants growing at O, E_O and E_W, and about 2 mg g^{-1} DM in plants growing at the W microsite. Relatively high CP concentrations correspond with earlier findings for shade grown forages (Kephart and Buxton 1993; Lin et al. 2001; Neel et al. 2004). Shade-grown orchardgrass nutritive value based on CP alone would be considered excellent; however, efficient CP utilization in the rumen depends on a readily fermentable source of energy (Hoover and Stokes 1990; Chiavarella et al. 2000).
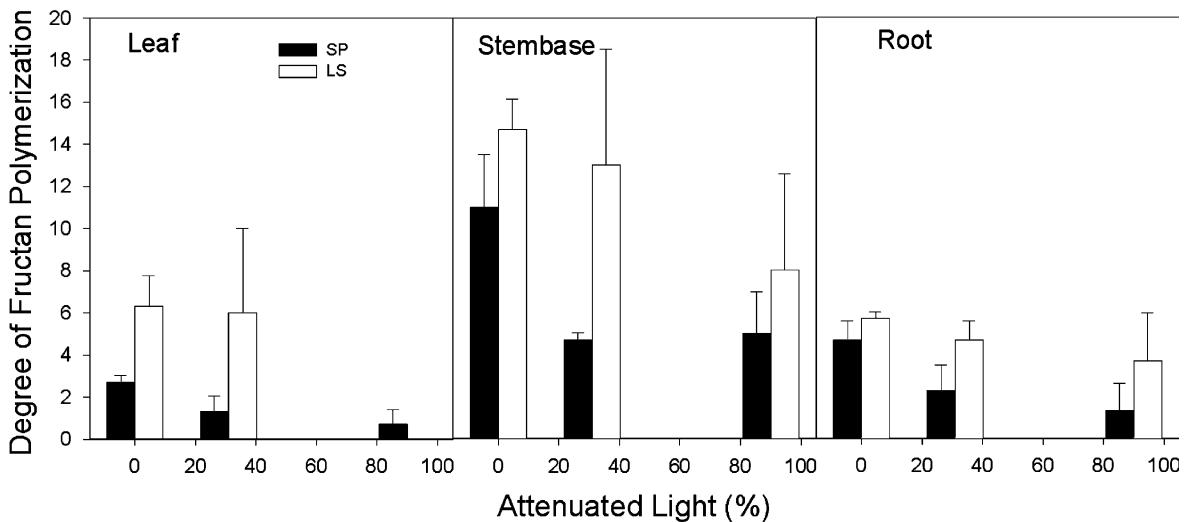

Figure 4. Degree of water-soluble carbohydrate polymerization (DP) for leaf, stembase and root as a function of microsite for spring (SP) and late-summer (LS) plantings. Values are mean and standard error of the mean of five harvests for each microsite of spring (SP) and three harvests for open (O) and edge-open (E_O), four for edge-woods (E_W) and five for woods (W) for late-summer (LS) planted orchardgrass.

Reduced herbage intake and depressed live-weight gain by sheep is associated with low irradiance. This is thought to occur because soluble carbohydrate concentrations, linked to herbage preference and livestock performance, are low (Chiavarella et al. 2000; Mayland et al. 2000), but depressed performance could be the results of a chemostatic effect related to high N or nitrate concentrations.

Relationship of carbon:nitrogen

The C:N quotient decreased in SP and LS plants as available light decreased (Figure 5). The lower C:N quotient for SP plant leaves was a function of greater N (Figure 6a). The same relative difference occurred for stembase C:N (Figure 5). The C:N relationship for roots was similar for SP and LS plantings. The amount of energy relative to nitrogen is as important as quantity of either class of compounds from the standpoint of rumen function (microbial metabolism) and N-use efficiency by the grazer.

Expressing the C:N quotient as TNC:CP shows that SP leaves were similar across a range of light conditions, but decreased substantially when comparing LS plants grown at O with those at W

sites (Figure 6c). The different responses for SP and LS plantings reflect characteristics associated with chronological or physiological maturity.

Feedstuff energy calculated as total digestible nutrients (TDN), allows expression of the energy:protein quotient on a per-unit and nutrient requirement basis. Estimates of forage TDN indicate high quality forage under all growing conditions (Figure 6b). The precise amount of energy relative to CP required for efficient rumen function and grazer response is not defined clearly. A TDN:CP range of 5.0–7.0 could meet animal and rumen micro-organism needs while allowing for variation in forage system management and seasonal growing conditions (NRC 1996; Moore et al. 1999). Values less than 5.0 clearly indicate excess herbage N, leading to questionable N-use efficiency. Grazing livestock might avoid forages with TDN:CP values less than 5.0 because of low TNC concentrations (Mayland et al. 2000).

The TDN:CP quotient declined as light attenuation increased for SP and LS plants (Figure 6d). The TDN:CP quotient for SP herbage declined from 4.7 (O) to 3.0 (W), because leaf TNC was similar and CP increased. In general, TDN:CP quotients for SP plants suggest that CP is excessive relative to energy when related to diet requirements for grazing livestock (e.g. beef cattle
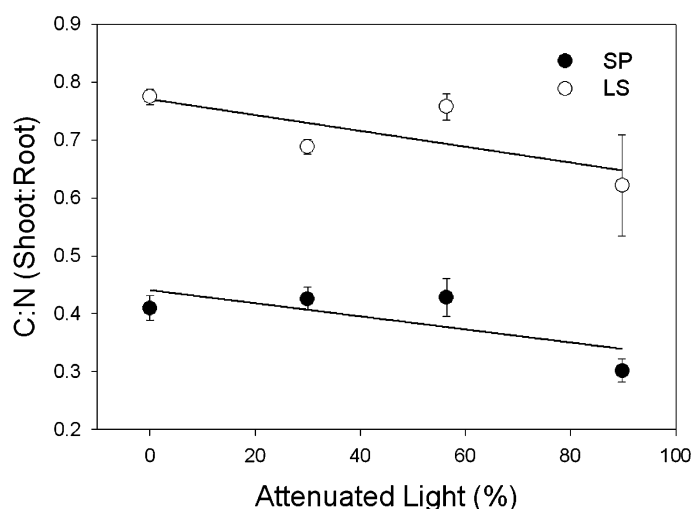

Figure 5. Carbon:nitrogen (C:N) quotient for plant components as a function microsite for spring (SP) and late-summer (LS) plants. Values are mean and standard error of the mean of five harvests for each microsite of spring (SP) and three harvests for open (O) and edge-open (E_O), four for edge-woods (E_W) and five for woods (W) for late-summer (LS) planted orchardgrass. Test $\beta_1 = 0$: SP, -0.0011 ns $r^2 = 0.51$; LS, -0.0014 ns $r^2 = 0.56$.
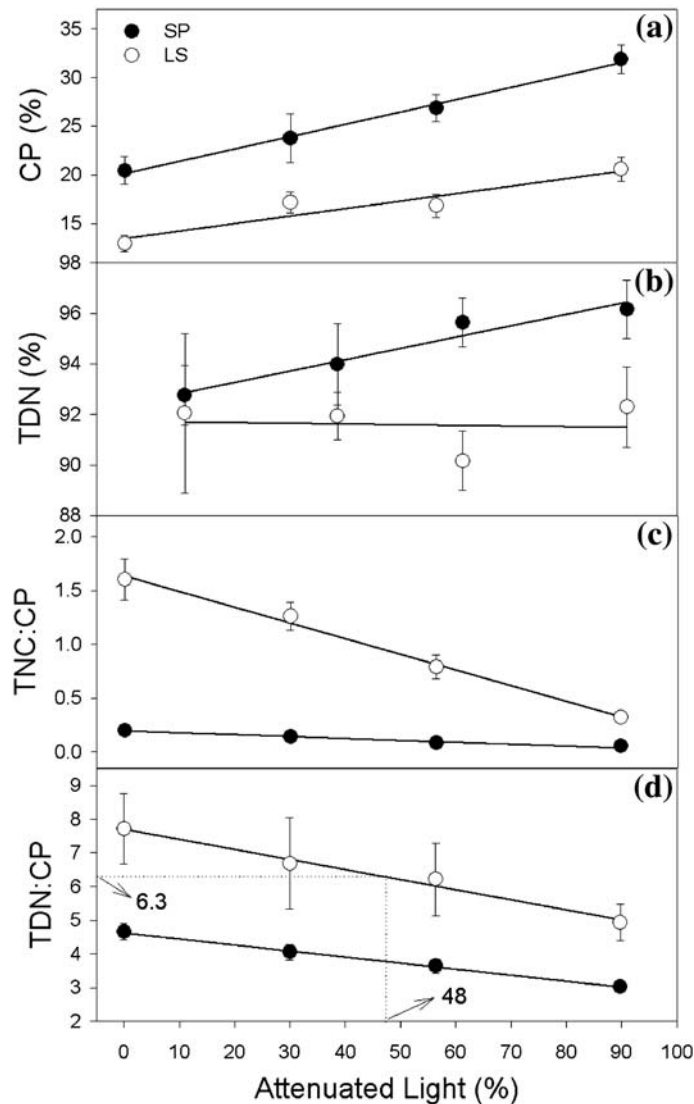
Figure 6. (a) Crude protein (CP) concentration as a function of microsite for spring (SP) and late-summer (LS) plantings. Values for parameters presented in Figure 6a–d are mean and standard error of the mean of five harvests for each microsite of spring (SP) and three harvests for open (O) and edge-open (E_O), four for edge-woods (E_W) and five for woods (W) for late-summer (LS) planted orchardgrass. Test $\beta_1 = 0$: SP, 0.1289** $r^2 = 0.99$; LS, 0.0783* $r^2 = 0.90$; (b) Total digestible nutrients (TDN), SP, 0.0398* $r^2 = 0.95$; LS, −0.0024 ns $r^2 = 0.01$; (c) Total nonstructural carbohydrate:crude protein (TNC:CP) quotient, SP, −0.0017* $r^2 = 0.97$; LS, −0.0146** $r^2 = 0.99$; (d) Total digestible nutrient:crude protein (TDN:CP), SP, −0.0169** $r^2 = 0.99$; LS, −0.0272 ns $r^2 = 0.87$.

(*Bos taurus*); NRC 1996). The TDN:CP of LS herbage ranged from about 7.8 (O) to 4.9 (W), representing a nutritional source that is more acceptable than SP herbage. The change in TDN:CP quotient of LS herbage was a product of decreasing TNC and increasing CP with respect to decreasing light availability and agrees with previous data on the nutritive value of shade-grown herbage (Deinum et al. 1968; Neel et al. 2004). A readily fermentable carbohydrate:digestible intake protein quotient of 2 or more is thought to optimize rumen microbe metabolism (Hoover and Stokes 1990). Our index based on TNC:CP ranged from 1.6 to 0.2, but may differ, since we use CP rather than digestible protein to compute the quotient.

Given our suggested TDN:CP range, SP plants had excessive N relative to energy irrespective of microsite, and LS plants grown at O sites contained substantially more energy relative to N. Consequently, SP forages could generate high rumen NH_3–N, which could depress intake, and excessive plasma urea nitrogen (PUN) leading to increased urinary N loss. Rumen microbes use plant carbohydrates (structural and nonstructural) as primary energy sources, whereas endproducts of microbial fermentation supply energy for the grazing ruminant (Chesson and Forsberg 1988). Energy requirements of rumen microorganisms might not be met when large amounts of herbage N occur relative to carbohydrate (Wallace and Cotta 1988). This ultimately impacts grazer energy balance because of the type and amount of fermentation end-products generated by the rumen biota. Animal performance was similar on open pasture and silvopasture despite superior nutritive value estimates for silvopasture forage (Neel et al. 2003). There are some tradeoffs in terms of nutritive value of shade-grown compared to open pasture herbage; increased protein content is accompanied by lower fiber and lignin concentrations (Kephart and Buxton 1993), lower TNC and reduced grazer preference. Shadegrown herbage use can be delayed somewhat during the growing season relative to open pasture, because fiber content is low and the typical advance in maturity and decline in herbage quality is slow. Since ruminant livestock derive energy from fibrous feedstuffs, estimate of the nutritive value of silvopasture herbage should include fractionation of the fibrous components (including lignin), development of energy component profiles and measurement of animal performance.

Summary

Silvopastoral components of grazing systems in the temperate eastern USA provide opportunity to extend the spatial boundaries of herbage production. This could help to diversify and increase forage-based livestock productivity on typical small-scale farms in the region, which are a mixture of woodlands and open pasture. Microsite conditions associated with light in silvopastoral agro-ecosystems create a range of herbage pro-

duction and dry matter allocation patterns that influence physiological responses and growth efficiencies of cool-temperate origin grasses. The patterns are reflected in nonstructural carbohydrate and N concentrations that ultimately influence nutritive value and grazing livestock performance. We found that orchardgrass yield and nutritive value were acceptable when not more than 45–50% light attenuation (as a function of shading by nearby trees) occurred relative to open pasture. Defoliation based on mean sward height resulted in varying numbers of harvests and regrowth intervals depending on microsite. Mean sward height (e.g. leaf elongation) used as an index for open pasture management might not apply for shaded sites, since leaf elongation is a response to low irradiance and not simply an inherent morphological characteristic of the plant.

Orchardgrass planted in spring and harvested in the year of planting, produced ample herbage mass (Belesky 2005a), but nutritive value measured in terms of TDN:CP was low because herbage N was excessive relative to energy. Nutritive value of plants sown late in a growing season and harvested in the subsequent growing season met theoretical ideal energy-protein quotients for efficient animal performance at all except O sites. Plants growing in full sunlight (O) had insufficient herbage N relative to energy. This suggests that nutritive value is improved by modest amounts of shade relative to plants growing in full sunlight, and that allowing cool temperate origin grasses to vernalize is beneficial in terms of nutritive value, as well as persistence (Belesky 2005a). The relationship of TNC:CP in orchardgrass reflected that of TDN:CP and might be an alternative way to evaluate nutritive value for cool-temperate grasses. Based on herbage composition, fertilizer needs for shaded sites might not be the same as those required for open pasture, since N concentrations in shade-grown plant tissues were relatively high with only modest N inputs. Small paddock experiments are underway that evaluate when grazing events should occur in hardwood silvopasture and compare traditional pasture to silvopasture in terms of livestock response, forage productivity, and nutritive value. Grazing duration and frequency in the paddock-scale experiments are based on findings obtained from the orchardgrass microplot experiments summarized in this paper. The energyprotein models derived from orchardgrass data are

being evaluated in terms of grazer blood chemistry to define N-use efficiency.

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